(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 229 599 A2**

(12) **EUROPEAN PATENT APPLICATION**

(21) Application number: 86850367.3

(51) Int. Cl.⁴: G01N 35/00

(22) Date of filing: 22.10.86

(30) Priority: 07.11.85 US 796093

(43) Date of publication of application: 22.07.87 Bulletin 87/30

(84) Designated Contracting States: DE FR GB SE

(71) Applicant: **BIFOK AB**
Box 124
S-191 22 Sollentuna(SE)

(72) Inventor: **Ruzicka, Jaromir**
Attemosevej 26
DK-2850 Holte(DK)
Inventor: **Hansen, Elo Harald**
Granasen 93
DK-2800 Lyngby(DK)

(74) Representative: **Rosenquist, Holger et al**
H Albihns Patentbyra AB Box 7664
S-103 94 Stockholm(SE)

(54) Sample introduction system for nonsegmented continuous flow analysis.

(57) Sample injection system for nonsegmented, continuous flow analysis comprising an inlet channel (2), an analyzer channel (3) and a carrier stream channel (4), connected in a confluence point (1), an aspirating pump (6) in the analyzer channel, a carrier stream pump (5) in the carrier stream channel with a higher capacity than the aspirating pump, and an analyzer (7).

In a first step prior to the sample injection, the carrier stream pump (5) forwards carrier stream to the confluence point (1), the aspirating pump (6) sucks carrier stream from the confluence point (1), and an outflow of carrier stream drains through the inlet channel (2).

In a second step, the carrier pump (5) is stopped, the inlet end (10) of the inlet channel (2) is brought in contact with the sample solution (14), and the aspirating pump (6) sucks sample into the inlet channel (2) and the analyzer channel (3).

In the third step, the carrier pump (5) is restarted, whereby the sample in the analyzer channel is aspirated into the analyzer (7), and all the sample in the inlet channel is pressed out into the waste (8).

The analyzer may be a flow-through detector, dialyzer, or any other apparatus for analysis.

The performance can also be made as a multichannel system for simultaneous analysis of several components in different analyzers.

In the above embodiments, the sample container must be connected and disconnected at precisely defined times. In order to avoid this, a second channel (17) can be added to the carrier pump (5) to make a double channel, the second channel (17) being connected to an outlet (16) from the inlet channel. The sample passes through the analyzer (7) and exits through the aspirating pump (6).

By instead connecting the second channel of the double channel pump to the analyzer channel (3) at a point between the analyzer (7) and the aspirating pump (6), the sample can be sucked in through the analyzer and after analysis be forced back the same way.

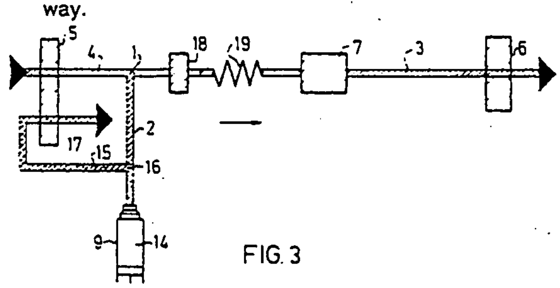

FIG. 3

EP 0 229 599 A2

Sample introduction system for nonsegmented continuous flow analysis

The successful operation of any flow injection analysis (FIA) system requires injection of a well-defined sample zone into the analyzer channel, where the zone disperses in a controlled manner on its way towards and through the detector to be measured upon (cf US Patents 4 022 575, 4 177 677, 4 224 033, 4 314 824, 4 227 973, and 4 315 754, and US Patent Applications 48 002, 296 256, 320 483, and 385 049). The injection devices designed for this purpose so far can be divided into two categories: (a) volume based injection; and (b) time based injection, or a combination thereof. In category (a) the injection is based on the physical entrapment of sample solution into a geometrically well-defined volumetric cavity and subsequent transfer (injection) of the thus formed sample zone into a nonsegmented carrier stream (cf the above-mentioned FIA monograph and US Patents 4 177 677 and 4 224 033). Category (b) is based on aspiration of sample solution at a constant flow rate for a fixed period of time into a well-defined section of a flow-through channel, from where the metered sample zone is injected into a nonsegmented carrier stream by a combination of hydrostatic and hydrodynamic forces -the so-called hydrodynamic injection procedure (cf US Patent Application 385 049).

Injection systems for gas chromatography are disclosed by Posposil, USP 4 464 940 for injection of gases. Such systems, however, cannot be used for incompressible liquids.

The purpose of the present invention is to describe a simplified and improved development of the hydrodynamic injection approach, which is based on the use of a confluence point at which a well-defined sample zone is formed by means of the alternate motion of sample and carrier streams.

Fig 1A-C, Fig 2, 3 and 4 show various embodiments of the hydrodynamic injection system according to the invention. The principle is best explained by reference to Figs 1A, 1B and 1C, where 1 is the confluence point; 2 is the inlet channel; 3 is the analyzer channel; 4 is the carrier stream channel; 5 is the carrier stream pumping device; 6 is the analyzer channel pumping device; 7 is the analyzer situated in the analyzer channel; 8 is the waste receptacle; 9 is the sample source; 10 is the inlet end of the inlet channel; 11 is the reservoir of the carrier stream fluid; 12 is the waste outlet for the analyzer channel; 13 is the carrier solution; 14 is the sample solution; and 15 is the injected sample zone. The operational cycle of the system comprises three sequential steps, each of which is represented in Fig 1A-C, details being as follows.

STEP 1 (Fig 1A), i.e. prior to injection of sample solution: Pump P1 5 pumps carrier stream solution 13 towards the confluence point 1 at a higher volumetric pumping rate than pump P2 6 which by its mode of action aspirates the liquid of the analyzer channel 3, that is, pumps away from the confluence point, resulting in a positive outflow of carrier stream solution through the inlet channel 2 and via 10 into waste 8, while sections 3 and 7 concurrently are filled with carrier stream solution flowing towards waste 12.

STEP 2 (Fig. 1B), i.e. sample aspiration: Pump P1 5 is stopped and the column of carrier stream solution 13 in the carrier stream channel 4 is held still, while pump P2 is maintained operating thereby aspirating sample solution 14 from container 9 which now has been moved into contact with inlet end 10.

STEP 3 (Fig 1C), i.e. sample injection: While pumping by P2 is maintained, pumping by pump P1 is resumed and sample source 9 is withdrawn. Thus, all sample solution 14 to the left of the confluence point 1 is forced in countercurrent fashion from the sample inlet into waste 8, while all sample solution in the form of an injected sample zone 15 to the right of the confluence point 1 is aspirated, followed by carrier stream solution 13, through the analyzer channel 3 and into the analyzer 7 for further treatment and measurement. As both pumps P1 and P2 continue pumping, all sample material is eventually expelled from the system either via the inlet channel 10 or via the Pump P2 6 channel 7, the analyzer 6 and the waste outlet 12, and the system is thus reestablished for the next sampling period, being now, in fact, in STEP 1 again.

While the explanation given above describes the principles of the new, proposed mode of injection, it should be noted that variations on this theme are possible without constraining the spirit of the invention. Thus, if a sample is too concentrated to be processed by the analyzer 7, an additional dilution may be executed at the confluence point 1 by decreasing -during the duration of STEP 2 -the pumping rate of the inflowing carrier stream below the aspiration rate of pump P2 6, instead of stopping P1 entirely. Furthermore, if the carrier stream would contain a reagent with which the sample solution should be mixed for the purpose of an intended chemical reaction, which is to be subsequently monitored in the analyzer 7, an effective mixing can thus be obtained during the passage of material through channel 3 to the analyzer 7. The analyzer 7 may be a flow-through detector, or may even comprise a system of channels, detectors,

dialyzer, or gas diffusion and pumping units -the only restriction being that the net inflow rate fulfills the conditions stipulated above, i.e. the flow rate through the analyzer 7 is governed solely by the aspiration flow rate of 6.

It is of interest to mention that the counter-current movement of liquid through channel 2 may be used with advantage if the sampled liquid contains particulate matter which has to be removed prior to automated assay. By placing a filtering device at position 10, particles may be retained on the filter during aspiration of the sample and discarded during the subsequent discharge period - (STEP 3).

It is important to emphasize that the repetitive movements of sample solution 14 and the inlet end 10 must be executed in harmony with the counter-current movement of liquid through channel 2 in such a way that under no circumstances air will enter the analyzer channel 3.

For operation of the injection system in that case where pump P1 is stopped during the sampling period (STEP 2), the injected sample volume S_v can be calculated from the stopped time interval T of pump P1 and the aspiration rate Q of pump P2 and by correcting for the expelled sample liquid contained within the inlet channel 2 of volume 1_v, i.e.:

$S_v = (T.Q) - 1_v$.

A modified process is illustrated in Fig. 2. The same reference numerals have been used for components corresponding to those in Fig. 1. In this embodiment the timing of the mechanical connection and disconnection of the sample container to and from the channel 2 must be precise.

The pumping rate for incoming carrier solution is designated x and y for outgoing analyzed sample and carrier solution, respectively. x is greater than y.

In STEP 1, the system is washed with carrier solution. Both pumps 5 and 6 are in operation and the sample channel 2 is opened freely to the drain.

In STEP 2, the pump 5 is stopped, the sample container 9 is connected and the sample 9 is drawn through the channel 2, and a predetermined quantity is drawn past the point of confluence 1.

In STEP 3, the pump 5 is started, the sample container 9 is disconnected and the carrier solution breaks the sample stream at the confluence point 1 and thus delimits the measured sample quantity which is then sucked through the channel 3 to the analyzer 7. In the channel 2, the remaining sample is forced out and replaced by carrier solution.

In the embodiment shown in Fig 3, the sample 14 need not be disconnected at any specific point in time. At a branch point 16 in the sample channel 2, there is a branch channel 15 which leads to the pump 17 and from there to the drain. The pump for the carrier solution is in the form of a double channel peristaltic pump 5,17. The pump rates for pumps 5, 6 and 17 are x, y and z, wherein z > x > y.

Since peristaltic pumps with a certain pulsation are used here, a filter 18 is placed in the channel 3 to prevent any air or particles from entering the analyzer 7.

In STEP 1, all of the pumps are operating, and the sample container 9 can be connected or disconnected. The pump 5 pumps carrier solution into the system, pump 6 pumps carrier solution and any sample residue out, and pump 17 pumps out a mixture of carrier solution and sample or air.

In STEP 2, the pump 5,17 is stopped, and pump 6 sucks sample into the channel 2 and a certain predetermined quantity of sample past the confluence point 1 in channel 3. The sample container 9 is connected during this step.

In STEP 3, the pump 5,17 is restarted, and pump 5 pumps carrier solution through the channel 4 into channels 3 and 2 forcing the measured sample quantity through the detector 7 where analysis takes place. The pump 17 pumps via branch point 16 and channel 15 out carrier solution from channel 2, and sample solution, if the sample container is still connected, or air, if it has been removed.

Fig 4 shows an embodiment where the sample is sucked into the coil and the analyzer, and after measuring is sucked back the same way. The pump rates are for 5, 6 and 17, x, y and z, respectively, wherein z > x > y.

In STEP 1, all of the pumps are operating, and the sample container can be connected. The channels are rinsed of previous samples by the carrier solution.

In STEP 2, the pump 5,17 is stopped while pump 6 continues to operate. The sample is sucked from the sample container 9 through the channel 2 past the point of confluence 1 through the coil 19 and the analyzer 7 and past the branch point 16.

The time interval for STEP 2 can also be adjusted so that only a portion of the sample passes through the analyzer 7.

In STEP 3, the pump 5,17 starts and carrier solution flows through channel 4 to the branch point 16, and from there a portion is sucked out to the drain by pump 6 while the rest forces the sample back through the analyzer 7 and the coil 19, past the confluence point 1, through the channel 15 and out to the drain. Since the pump rate z is greater than x -y, sample 14, or air if the sample container is removed, will be sucked up via the channel 2, the confluence point 1, and in mixture with the carrier solution through channel 15 and pump 17 to the drain.

The filter in this embodiment can be left out or placed after the coil 19.

Reagent can be added together with the carrier flow or separately through a coupled dozing device.

The advantages of the new injection system may be summarized as follows:

(1) No moving parts in contact with the handled liquids.

(2) Lesser mechanical complexity than in any valving system.

(3) Ideally suited for microminiaturization.

(4) Ideally suited for integration into microconduits, which without any mechanical moving parts are easy to make, inexpensive and disposable.

(5) The sample volume -and the sample dispersion -is adjusted by timing sequences rather than by adjusting the volume of a cavity in a valve, that is, electronic computer control rather than mechanical control of the dispersion is exerted.

(6) Less complex than "classical" hydrodynamic injection -only two pumping tubes are required, and the streams do not have to be balanced.

(7) Countercurrent operation of the inlet part of the system, and/or the detector, allowing clean-up and/or filtration.

Claims

1. A sample injection system for nonsegmented continuous flow analysis (FIA), characterized in the use of a single confluence point (1) connecting three channels through which liquids may flow, wherein the first of said channels, the inlet channel (2), which alternately is contacting a liquid sample source (14) and a waste outlet (8), is operated in a countercurrent fashion by means of the intermittent action of two pumping devices (5,6,17), one of said pumping devices (6) capable of aspirating a stream of liquid through the inlet channel (2) and via said confluence point (1) into the second of said channels (3), in which said second channel, the analyzer channel (3), a detection device (7) for analyzing said flowing stream is situated, and the second one (5) of said pumping devices capable of forwarding a stream of liquid through the third of said channels, the carrier channel (4), towards and past said confluence point (1), the two pumping devices being operated in such a way that the flow rate in said carrier channel (4) is higher than the aspiration rate of said first pumping device (6) situated in said analyzer channel.

2. Sample injection system according to Claim 1, characterized in that said intermittent operation of flow of liquids is executed by first stopping the flow of liquid in said carrier channel (4) while the aspiration of sample fluid is effectuated through said inlet carrier channel when a desired volume of said sample fluid has flown past said confluence point (1) and into said analyzer channel (3).

3. Sample injection system according to Claims 1 and 2, characterized in that the length of the stop time of the flow of liquid in said carrier channel (4) can be selected in such a manner that it allows, together with the aspiration flow rate within said analyzer channel (3), the exact selection of the volumetric amount of said sample solution to be introduced into said analyzer channel in the form of a well-defined zone.

4. Sample injection system according to Claims 1 and 3, characterized in that the flow rate - (x) of the stream of liquid in said carrier channel - (4) is temporarily reduced or stopped below the flow rate of the aspiration flow rate (y) of liquid in said analyzer channel while a sample source is in contact with said inlet channel, and a higher rate of flow (x) in the carrier channel is resumed when a desired volume of the thus diluted sample fluid has flown past said confluence point (1) and into the analyzer channel.

5. Sample injection system according to anyone of the above claims, characterized in that said sample source (9) is a container or a continuously flowing stream, said sample sources being periodically manipulated so that the sample fluid comes into contact with the end of said inlet channel (2) only during the sample aspiration period and is removed from the end of said inlet channel during the sample inlet discharge period, which corresponds to the time sequence during which the pumping device (5) situated in said carrier channel (4) is operating at a volumetric pumping rate (x) exceeding that (y) of said pumping device (6) situated in said analyzer channel (3).

6. Sample injection system according to Claim 1, characterized in several analyzer channels (3) from the confluence point (1), each with a separate analyzer (7) to enable simultaneous determination of different substances.

7. Sample injection system according to anyone of Claims 1-5, characterized in that the second pump is in the form of a double channel pump - (5,17), the first channel (5) being arranged to pump carrier solution through carrier channel (4) to the branch point (16) and the second channel to aspirate carrier solution and/or air through a channel - (15) from a branch point (16) in the inlet channel - (2), the pump rate for pumps (5,6,17) being x, y and z, respectively, where z x y, so that when the double channel pump (5,17) is stopped, sample - (14) will be sucked in through the inlet channel (2), the confluence point (1), a filter (18) a coil (19), the analyzer channel (3) and the analyzer (7) by pump

(6), which can be operating continually, and when the double channel pump (5, 17) is started, the part (5) is arranged to pump in carrier solution via the confluence point (1) out into the analyzer channel - (3) and the inlet channel (2), while the pump (17) sucks from the inlet channel (2) carrier solution and sample solution and/or air depending on whether the sample container is removed or not.

8. Sample injection system according to anyone of Claims 1-5, characterized in that the second pump is in the form of a double channel pump - (5,17) one part (5) of which being arranged to pump in carrier solution through carrier channel (4) to branch point (16) and the other part (17) to aspirate carrier solution and sample solution or air from branch point (16) in the analyzer channel (3) through the analyzer (7), the coil (19) and the filter (18), if any, to the confluence point (1), where additional sample solution and/or air is sucked in from the inlet channel (2), and further through the channel (15) to the drain, the pump rates for the pumps (5,6,17) being x, y and z, respectively, where z x y, so that when the double channel pump (5,17) is stopped, sample (14) will be sucked in through the inlet channel (2), the confluence point (1), the filter (18), the coil (19) and the analyzer (7) to the branch point (16) by pump (6), which can be operating continually, and when the double channel pump (5,17) is started, the sample will be led to the drain, the channels will be washed clean and filled with carrier solution.

9. Sample injection system according to anyone of Claims 1-8, characterized by a device for dozing reagent to the system for reaction with the sample.

10. Method for injecting a sample at nonsegmented continuous flow analysis, based on a confluence point (1) connecting three channels, an inlet channel (3) for the sample (14) with a movable source (9) for the sample, a carrier channel (4) with a pump (5) with the rate x and a source for the carrier solution (13), and an analyzer channel (3) with an aspirating pump (6) with a rate y and a detector (7) for determination of substances in the sample, characterized in that the pumping rate $x > y$ and in STEP 1 prior to the injection, the carrier pump (5) pumps carrier solution to the confluence point (1), the analyzer pump (6) aspirates part of the carrier solution and the remainder goes to the waste through the inlet channel (2), in STEP 2 the sample source (9) is contacted with the inlet channel (2), the carrier pump (5) is stopped and the analyzer pump (6) aspirates sample (14) through the inlet channel (2) past the confluence point (1) and into the analyzer channel (3) in a predetermined amount, and in STEP 3 the carrier pump (5) is started again, and the sample amount in the analyzer channel (3) after the confluence point (1) is aspirated into the detector (7) and determined.

11. Method according to Claim 10, characterized in that the carrier pump (5) is made as a two-channel pump (5,17) and that an additional channel (15) is arranged from a branch point (16) in the inlet channel (2) to the pump (17) with capacity z, where $z > x > y$, and that in STEP 1 all the pumps are operating and the sample container can either be connected or disconnec ted, the carrier pump - (5) pumping carrier solution into the system, the analyzer pump (6) sucking out carrier solution and the additional pump (17) pumping out a mixture of carrier solution and sample or air, in STEP 2 the sample container is connected, the two-channel pump is stopped and the analyzer pump (6) sucks sample into the inlet channel (2) and a certain predetermined amount past the confluence point - (1) and into the analyzer channel (3), and in STEP 3 the two-channel pump (5,17) is started again and the carrier solution is forced past the confluence point (1) cutting off the measured sample amount which is sucked through the detector (7) and analyzed there.

12. Method according to Claim 10, characterized in that the carrier pump is made as a two-channel pump (5,15), that the carrier channel (4) extends from the pump (5) to the branch point (16) after the detector (7) in the analyzer channel (3) and that the channel (15) extends from the confluence point (1) to the additional pump (17), whereby in STEP 2 the sample passes through the detector (7) and is analyzed, and in STEP 3 the sample passes back through the detector to the confluence point (1) and from there out through the channel (15).

13. Method according to Claim 12, characterized in that a complete curve of the sample gradient is taken in the detector.

14. Method according to anyone of Claims 10-13, characterized in that reagent is dozed into the system for reaction with the sample.

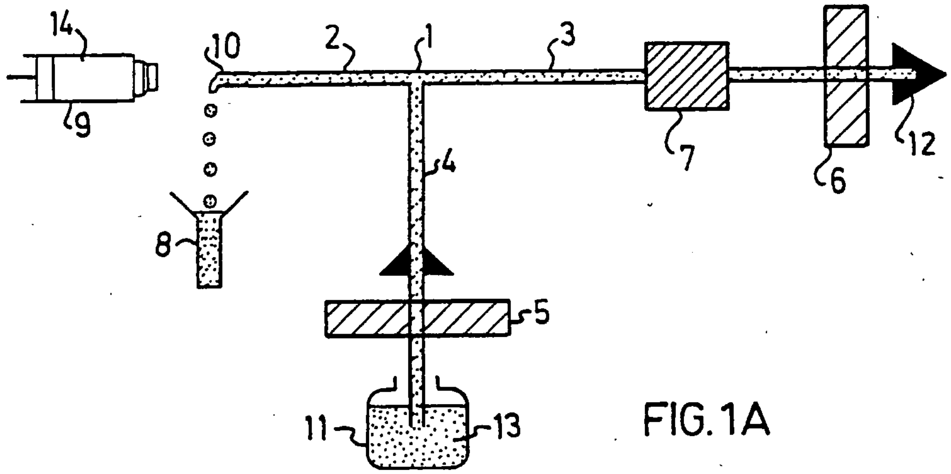

FIG. 1A

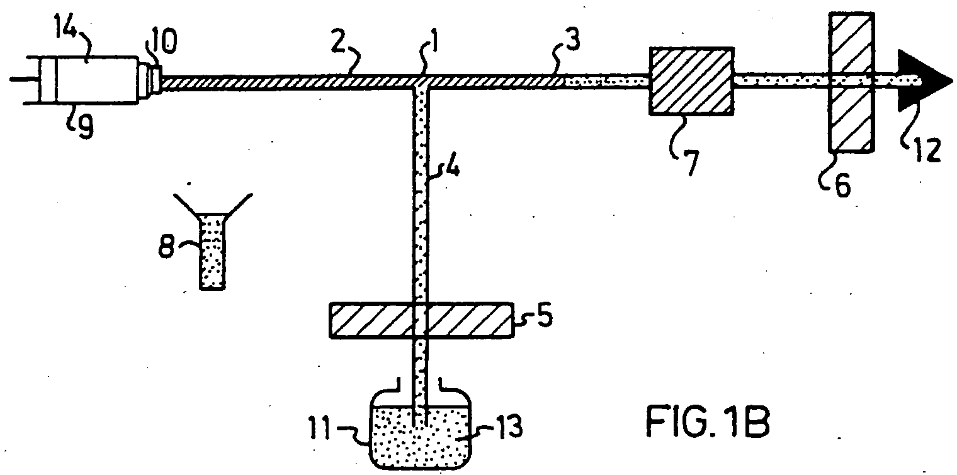

FIG. 1B

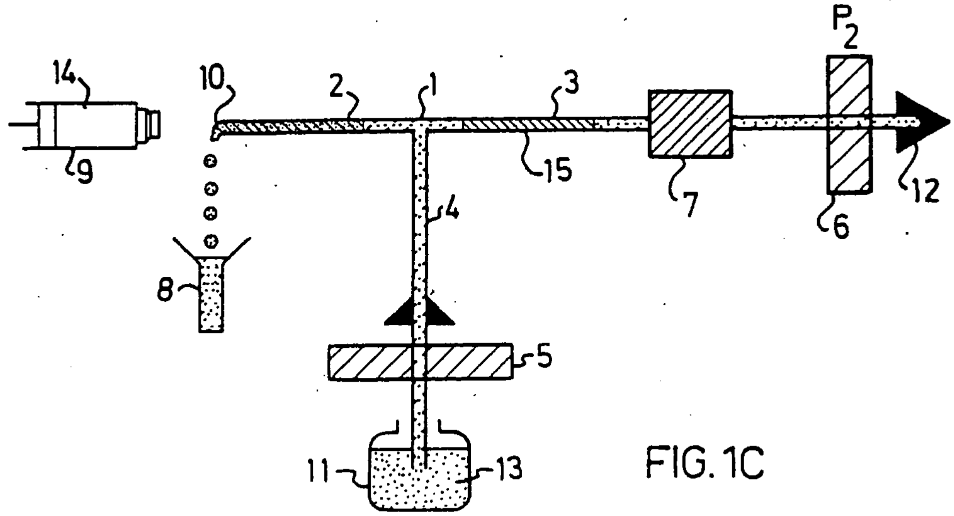

FIG. 1C

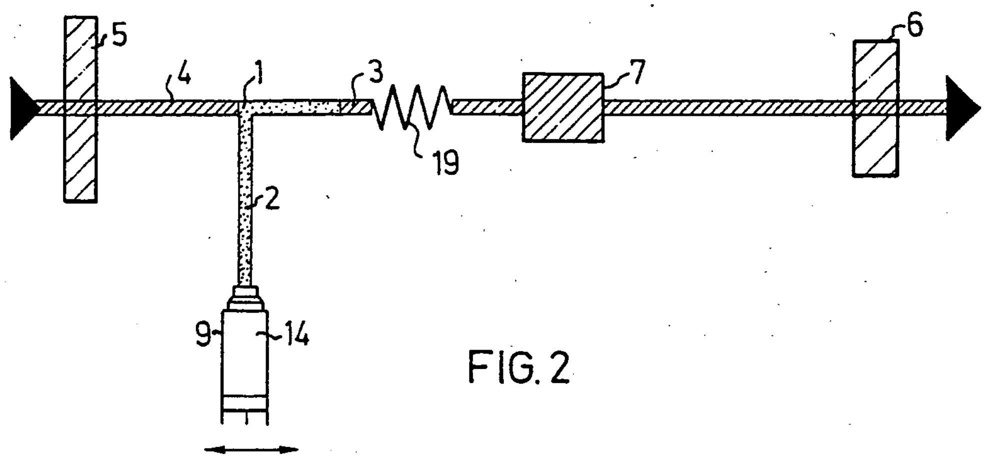

FIG. 2

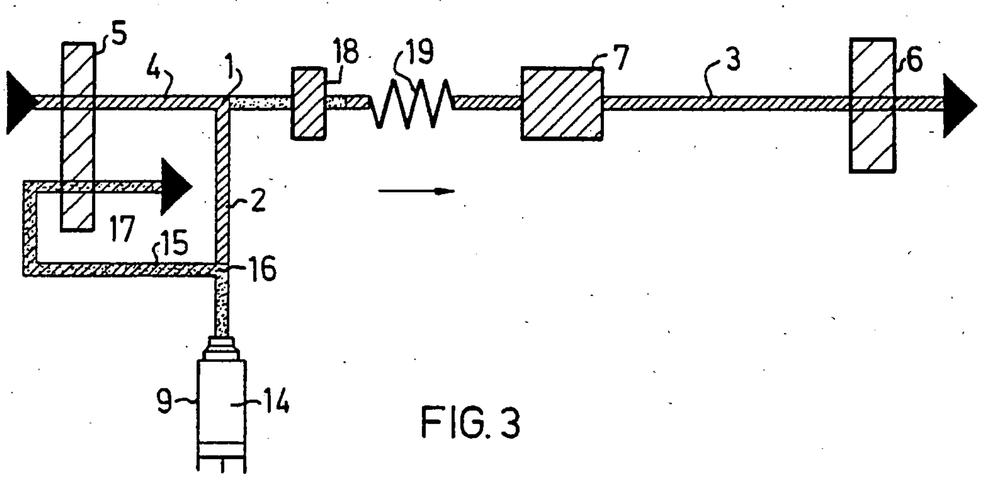

FIG. 3

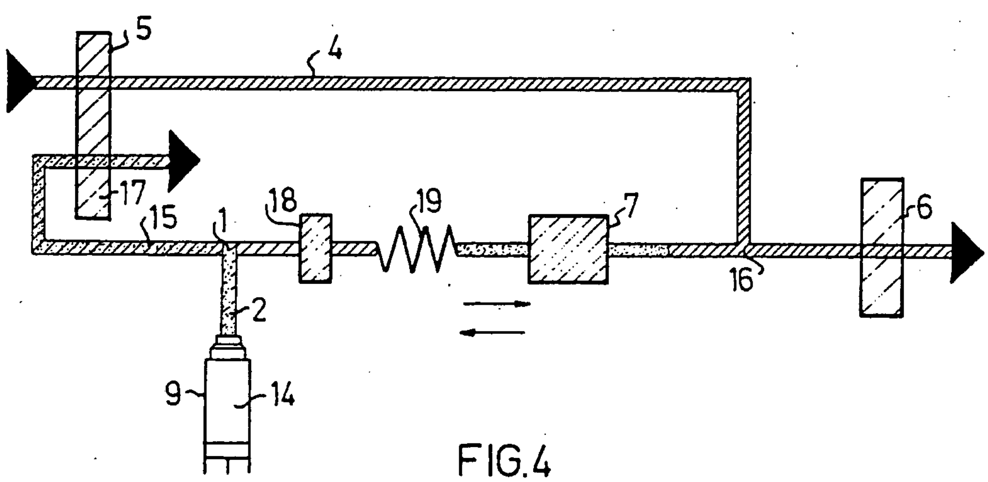

FIG. 4